



Express Mail Label No. EL902309562US

PATENT  
Attorney Docket No. STK-008CN

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S): Oppermann et al.  
SERIAL NO.: 09/754,831 GROUP NO.: 1647  
FILING DATE: January 3, 2001 EXAMINER:  
TITLE: Osteogenic Devices

Box Missing Parts  
Assistant Commissioner for Patents  
Washington, D.C. 20231

**PRELIMINARY AMENDMENT**

Sir:

Applicants respectfully request entry of this Preliminary Amendment in the above-identified application. A Sequence Listing is submitted herewith in computer-readable form and paper form. A paper copy of the Sequence Listing is attached herewith for the Examiner's convenience. The claims and the specification are amended to insert sequence identifiers into the application. Applicants also respectfully request entry of the Sequence Listing in the above-identified application. No new matter is introduced.

The Sequence Listing and the present Preliminary Amendment are responsive to the Notice to File Missing Parts of Nonprovisional Application, mailed from the Patent Office on March 8, 2001.

Please make the following changes before examining the patent application identified above.

09/754,831-091001

## In the Claims

Please cancel claims 1-45, 50-80 without prejudice, and amend claims 46-49 to read as follows. A marked up version of the amended claims is attached at the end of this Preliminary Amendment.

46. (Amended) A DNA sequence encoding an amino acid sequence sufficiently duplicative of that of the sequence encoded by the gene of Figure 1A (SEQ ID NO: 40) such that said encoded sequence induces bone or cartilage formation when implanted in a mammal in association with a matrix.

47. (Amended) The DNA of claim 46 encoding the same amino acid sequence as the gene set forth in Figure 1A (SEQ ID NO: 40).

48. (Amended) The DNA sequence of claim 46 encoding:

```

1           10           20           30           40
OP1      LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAPLNS
           50           60           70
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
           80           90          100
ISVLYFDDSSNVILKKYRNMVVRACGCH (SEQ ID NO: 39).
```

49. (Amended) The DNA sequence of the claim 46 encoding:

```

                                           -5
                                           HQRQA
1           10           20           30           40
OP1      CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAPLNS
           50           60           70
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
           80           90          100
ISVLYFDDSSNVILKKYRNMVVRACGCH (SEQ ID NO: 9).
```

T00T60" TBS4260

In the Specification:

where the letters indicate the amino acid residues of standard single letter code, and the Xs represent amino acid residues. Preferred amino acid sequences within the foregoing generic sequences are (SEQ ID NO: 5 and SEQ ID NO: 6, respectively):

Please replace the second full paragraph on page 11, line 8, with the following paragraph.

Particular useful sequences include Vgl (SEQ ID NO: 7), DPP (SEQ ID NO: 8), OP1 (SEQ ID NO: 9), CBMP-2a (SEQ ID NO: 10), CBMP-2b (SEQ ID NO: 11), CBMP-3 (SEQ ID NO: 12), COP1 (SEQ ID NO: 13), COP3 (SEQ ID NO: 14), COP4 (SEQ ID NO: 15), and COP16 (SEQ ID NO: 16):

Please replace the second full paragraph on page 14, line 5, with the following paragraph.

Analysis of digestion fragments indicate that the native 30 kD osteogenic protein contains the following amino acid sequences (question marks indicate undetermined residues):

- (1) S-F-D-A-Y-Y-C-S-G-A-C-Q-F-P-M-P-K (SEQ ID NO: 17);
- (2) S-L-K-P-S-N-Y-A-T-I-Q-S-I-V (SEQ ID NO: 18);
- (3) A-C-C-V-P-T-E-L-S-A-I-S-M-L-Y-L-D-E-N-E-K (SEQ ID NO: 19);
- (4) M-S-S-L-S-I-L-F-F-D-E-N-K (SEQ ID NO: 20);
- (5) S-Q-E-L-Y-V-D-F-Q-R (SEQ ID NO: 21);
- (6) F-L-H-C-Q-F-S-E-R-N-S (SEQ ID NO: 22);
- (7) T-V-G-Q-L-N-E-Q-S-S-E-P-N-I-Y (SEQ ID NO: 23);
- (8) L-Y-D-P-M-V-V (SEQ ID NO: 24);
- (9) V-G-V-V-P-G-I-P-E-P-C-C-V-P-E (SEQ ID NO: 25);
- (10) V-D-F-A-D-I-G (SEQ ID NO: 26);
- (11) V-P-K-P-C-C-A-P-T (SEQ ID NO: 27);
- (12) I-N-I-A-N-Y-L (SEQ ID NO: 28);
- (13) D-N-H-V-L-T-M-F-P-I-A-I-N (SEQ ID NO: 29);
- (14) D-E-Q-T-L-K-K-A-R-R-K-Q-W-I-?-P (SEQ ID NO: 30);
- (15) D-I-G-?-S-E-W-I-I-?-P (SEQ ID NO: 31);
- (16) S-I-V-R-A-V-G-V-P-G-I-P-E-P-?-?-V (SEQ ID NO: 32);
- (17) D-?-I-V-A-P-P-Q-Y-H-A-F-Y (SEQ ID NO: 33);
- (18) D-E-N-K-N-V-V-L-K-V-Y-P-N-M-T-V-E (SEQ ID NO: 34);
- (19) S-Q-T-L-Q-F-D-E-Q-T-L-K-?-A-R-?-K-Q (SEQ ID NO: 35);
- (20) D-E-Q-T-L-K-K-A-R-R-K-Q-W-I-E-P-R-N-?-A-R-R-Y-L (SEQ ID NO: 36);
- (21) A-R-R-K-Q-W-I-E-P-R-N-?-A-?-R-Y-?-?-V-D (SEQ ID NO: 37); and

(22) R-?-Q-W-I-E-P-?-N-?-A-?-?-Y-L-K-V-D-?-A-?-?-G (SEQ ID NO: 38).

Please replace the second full paragraph on page 16, line 5, with the following paragraph.

The consensus DNA sequences are also useful as probes for extracting genes encoding osteogenic protein from genomic and cDNA libraries. One of the consensus sequences has been used to isolate a heretofore unidentified genomic DNA sequence, portions of which when ligated encode a protein having a region capable of inducing endochondral bone formation. This protein, designated OP1, has an active region having the sequence set forth below (SEQ ID NO: 39 and SEQ ID NO: 9, respectively).

Please replace the fifth full paragraph on page 16, line 29, with the following paragraph.

Fig. 1A discloses the genomic DNA sequence of OP1 (SEQ ID NO: 40).

Please replace the second full paragraph on page 18, line 7, with the following paragraph.

FIGURE 1A represents the nucleotide sequence of the genomic copy of osteogenic protein "OP1" gene (SEQ ID NO: 40). The unknown region between 1880 and 1920 actually represents about 1000 nucleotides;

Please replace the third full paragraph on page 18, line 11, with the following paragraph.

FIGURE 1B is a representation of the hybridization of the consensus gene/probe (SEQ ID NO: 41) to the osteogenic protein "OP1" gene (SEQ ID NO: 42);

Please replace the third full paragraph on page 20, line 14, with the following paragraph.

FIGURE 13 is a schematic representation of the DNA sequence (SEQ ID NO: 43) and corresponding amino acid sequence (SEQ ID NO: 44) of a consensus gene/probe for osteogenic protein (COPO);

Please replace the second full paragraph on page 21, line 7, with the following paragraph.

FIGURE 18 is a comparison of the amino acid sequence of various osteogenic proteins to those of the TGF-beta family. COP1 (SEQ ID NO: 13), COP3 (SEQ ID NO: 14), COP4 (SEQ ID NO: 15), COP5 (SEQ ID NO: 1), and COP7 (SEQ ID NO: 2) are a family of analogs of synthetic osteogenic proteins developed from the consensus gene that was joined to a leader protein via a hinge region having the sequence D-P-N-G (SEQ ID NO: 45) that permitted chemical cleavage at the D-P site (by acid) or N-G (by hydroxylamine) resulting in the release of the analog protein; VGI (SEQ ID NO: 7) is a Xenopus protein, DPP (SEQ ID NO: 8) is a Drosophila protein; OP1 (amino acids 6-x of SEQ ID NO: 9) is a native osteogenic protein; CBMP2a (SEQ ID NO: 10) and 2b (SEQ ID NO: 11), and CBMP3 (SEQ ID NO: 12) are subparts of proteins disclosed in PCT application 087/01537; beta-Inhibin a) is shown in SEQ ID NO: 46, beta-Inhibin b) is shown in SEQ ID NO: 47, TGF-beta 1 is shown in SEQ ID NO: 48, TGF-beta 2 is shown in SEQ ID NO: 49, TGF-beta 3 is shown in SEQ ID NO: 50; MIS (SEQ ID NO: 51) is Mullerian inhibitory substance; alpha-Inhibin is shown in SEQ ID NO: 52; and "consensus choices" represent various substitutions of amino acids that may be made at various positions in osteogenic proteins;

Please replace the second full paragraph on page 22, line 4, with the following paragraph.

FIGURE 21B is the DNA sequence (SEQ ID NO: 53) and amino acid sequence (SEQ ID NO: 54) comprising a modified trp-LE leader, two Fb domains of protein A, an ASP-PRO cleavage site, and the COP5 sequence;

Please replace the second paragraph starting on page 47, line 9, and ending on page 48, line 4, with the following paragraph.

Various of the peptide fragments produced using the foregoing procedures have been analyzed in an automated amino acid sequencer (Applied Biosystems 470A with 120A on-line PTH analysis). The following sequence data has been obtained:

- (1) S-F-D-A-Y-Y-C-S-G-A-C-Q-F-P-M-P-K (SEQ ID NO: 17);
- (2) S-L-K-P-S-N-Y-A-T-I-Q-S-I-V (SEQ ID NO: 18);

- (3) A-C-C-V-P-T-E-L-S-A-I-S-M-L-Y-L-D-E-N-E-K (SEQ ID NO: 19);  
 (4) M-S-S-L-S-I-L-F-F-D-E-N-K (SEQ ID NO: 20);  
 (5) S-Q-E-L-Y-V-D-F-Q-R (SEQ ID NO: 21);  
 (6) F-L-H-C-Q-F-S-E-R-N-S (SEQ ID NO: 22);  
 (7) T-V-G-Q-L-N-E-Q-S-S-E-P-N-I-Y (SEQ ID NO: 23);  
 (8) L-Y-D-P-M-V-V (SEQ ID NO: 24);  
 (9) V-G-V-V-P-G-I-P-E-P-C-C-V-P-E (SEQ ID NO: 25);  
 (10) V-D-F-A-D-I-G (SEQ ID NO: 26);  
 (11) V-P-K-P-C-C-A-P-T (SEQ ID NO: 27);  
 (12) I-N-I-A-N-Y-L (SEQ ID NO: 28);  
 (13) D-N-H-V-L-T-M-F-P-I-A-I-N (SEQ ID NO: 29);  
 (14) D-E-Q-T-L-K-K-A-R-R-K-Q-W-I-?-P (SEQ ID NO: 30);  
 (15) D-I-G-?-S-E-W-I-I-?-P (SEQ ID NO: 31);  
 (16) S-I-V-R-A-V-G-V-P-G-I-P-E-P-?-?-V (SEQ ID NO: 32);  
 (17) D-?-I-V-A-P-P-Q-Y-H-A-F-Y (SEQ ID NO: 33);  
 (18) D-E-N-K-N-V-V-L-K-V-Y-P-N-M-T-V-E (SEQ ID NO: 34);  
 (19) S-Q-T-L-Q-F-D-E-Q-T-L-K-?-A-R-?-K-Q (SEQ ID NO: 35);  
 (20) D-E-Q-T-L-K-K-A-R-R-K-Q-W-I-E-P-R-N-?-A-R-R-Y-L (SEQ ID NO: 36);  
 (21) A-R-R-K-Q-W-I-E-P-R-N-?-A-?-R-Y-?-?-V-D (SEQ ID NO: 37); and  
 (22) R-?-Q-W-I-E-P-?-N-?-A-?-?-Y-L-K-V-D-?-A-?-?-G (SEQ ID NO: 38).

Please replace Table 6 page 54-55, with the following Table 6.

**TABLE 6**

<u>protein</u>	<u>amino acid sequence</u>	<u>homology</u>
(BOP) SEQ ID NO: 55	SFDAYYCSGACQFPS	(9/15 matches)
(DDP) SEQ ID NO: 56	GYDAYYCHGKCPFFL	
(BOP) SEQ ID NO: 55	SFDAYYCSGACQFPS	(6/15 matches)
(Vgl) SEQ ID NO: 57	GYMANYCYGECPYPL	
(BOP) SEQ ID NO: 55	SFDAYYCSGACQFPS	(5/15 matches)
(inhibin) SEQ ID NO: 58	GYHANYCEGECPSHI	

(BOP) SEQ ID NO: 55                      SFDAYYCSGACQFPS  
   \* \* \* \* \*                      (4/15 matches)  
(TGF-beta) SEQ ID NO: 59                      GYHANFCLGPCPYIW

(BOP) SEQ ID NO: 60                      K/RACCVPTELSAISMLYLDEN  
   \*\*\*\*\* \* \* \* \* \*                      (12/20 matches)  
(Vgl) SEQ ID NO: 61                      LPCCVPTKMSPISMLFYDNN

(BOP) SEQ ID NO: 60                      K/RACCVPTELSAISMLYLDEN  
   \* \* \* \* \* \* \* \* \* \* \*                      (12/20 matches)  
(inhibin) SEQ ID NO: 62                      KSCCVPTKLRPMSMLYYDDG

(BOP) SEQ ID NO: 60                      K/RACCVPTELSAISMLYLDE  
   \*\*\*\*\* \* \*                      (6/19 matches)  
(TGF-beta) SEQ ID NO: 63                      APCCVPQALEPLPIVYYVG

(BOP) SEQ ID NO: 60                      K/RACCVPTELSAISMLYLDEN  
   \*\*\*\*\* \* \* \* \* \*                      (12/20 matches)  
(DPP) SEQ ID NO: 64                      KACCVPTQLDSVAMLYLNDQ

(BOP) SEQ ID NO: 65                      LYVDF  
   \*\*\*\*\*                      (5/5 matches)  
(DPP) SEQ ID NO: 66                      LYVDF

(BOP) SEQ ID NO: 65                      LYVDF  
   \*\*\* \*                      (4/5 matches)  
(Vgl) SEQ ID NO: 67                      LYVEF

(BOP) SEQ ID NO: 65                      LYVDF  
   \*\* \* \*                      (4/5 matches)  
(TGF-beta) SEQ ID NO: 68                      LYIDF

(BOP) SEQ ID NO: 65                      LYVDF  
   \* \*                      (2/4 matches)  
(inhibin) SEQ ID NO: 69                      FFVSF

-  
\*-match

2007-09-24 14:54:50



Please replace the second paragraph starting on page 57, line 14, and ending on page 58, line 27, with the following paragraph.

Southern blot analysis of lambda #13 DNA showed that an approximately 3kb BamHI fragment hybridized to the probe. (See Fig. 1B). This fragment was isolated and subcloned into a bluescript vector (at the BamHI site). The clone was further analyzed by Southern blotting and hybridization to the COP0 probe. This showed that a 1kb (approx.) EcoRI fragment strongly hybridized to the probe. This fragment was subcloned into the EcoRI site of a bluescript vector, and sequenced. Analysis of this sequence showed that the fragment encoded the carboxy terminus of a protein, named osteogenic protein-1 (OP1). The protein was identified by amino acid homology with the TGF-beta family. For this comparison cysteine patterns were used and then the adjacent amino acids were compared. Consensus splice signals were found where amino acid homologies ended, designating exon intron boundaries. Three exons were combined to obtain a functional TGF-beta-like domain containing seven cysteines. Two introns were deleted by looping out via primers bridging the exons using the single stranded mutagenesis method of Kunkel. Also, upstream of the first cysteine, an EcoRI site and an asp-pro junction for acid cleavage were introduced, and at the 3' end a PstI site was added by the same technique. Further sequence information (penultimate exon) was obtained by sequencing the entire insert. The sequencing was done by generating a set of unidirectionally deleted clones (Ozkaynak, E., and Putney, S.: Biotechniques, 5, 770-773, 1987). The obtained sequence covers about 80% of the TGF-beta-like region of OP1 and is set forth in FIG. 1A (SEQ ID NO: 40). The complete sequence of the TGF-beta like region was obtained by first subcloning all EcoRI generated fragments of lambda clone #13 DNA and sequencing a 4kb fragment that includes the first portion of the TGF-beta like region (third exon counting from end) as well as sequences characterized earlier. The gene on an EcoRI to PstI fragment was inserted into an *E. coli* expression vector controlled by the trp promoter-operator to produce a modified trp LE fusion protein with an acid cleavage site. The OP1 gene encodes amino acids corresponding substantially to a peptide found in sequences of naturally sourced material. The amino acid sequence of what is believed to be its active region is set forth below (SEQ ID NO: 39):

Please replace the first full paragraph on page 59, line 1, with the following paragraph.

A longer active sequence is (SEQ ID NO: 9):

Please replace the third paragraph starting on page 61, line 27, and ending on page 62, line 28, with the following paragraph.

It was noted, for example, that DPP from drosophila, VG1 from Xenopus, the TGF beta family of proteins, and to a lesser extent, alpha and beta inhibins, had significant homologies with certain of the sequences derived from the naturally sourced OP product. (Fig. 18.) Study of these proteins led to the realization that a portion of the sequence of each had a structural similarity observable by analysis of the positional relationship of cysteines and other amino acids which have an important influence on three dimensional protein conformation. It was noted that a region of these sequences had a series of seven cysteines, placed very nearly in the same relative positions, and certain other amino acids in sequence as set forth below (SEQ ID NO: 4):

```
      10      20      30      40      50
CXXXLXVFXDXGWXXWXXXPXGXXAXYCXGXCCPXXXXXXXXNHAXX
      60      70      80      90     100
QXXVXXNXXXPXXCCPXXXXXXXXLXXXXXXXXVXLXXYXXMXVXXCXCX
```

wherein each X independently represents an amino acid. Expression experiments with constructs patterned after this template amino acid sequence showed activity occurred with a shorter sequence having only six cysteines (SEQ ID NO: 3):

```
      10      20      30      40      50
LXVFXDXGWXXWXXXPXGXXAXYCXGXCCPXXXXXXXXNHAXX
      60      70      80      90     100
QXXVXXNXXXPXXCCPXXXXXXXXLXXXXXXXXVXLXXYXXMXVXXCXCX
```

wherein each X independently represents an amino acid. Within these generic structures are a multiplicity of specific sequences which have osteogenic or chondrogenic activity. Preferred structures are those having the amino acid sequence (SEQ ID NO: 6):

```
      10      20      30      40      50
CKRHPLYVDFRDVGWNDWIVAPPGYHAFYCHGECFPFLADHLNSTNHAIV
```

```

RRRS K S S L QE VIS E FD Y E A AY MPESMKAS VI
KE F E K I DN L N S Q ITK F P TL
Q A S K

60 70 80 90 100
QTLVNSVNP GKIPKACCVPT ELSAISM LYL DENENVVLK NYQDMVVEGCGCR
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H
RF T S K DPV V Y N S H RN RS
N S K P E

```

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be used. Novel active proteins also are defined by amino acid sequences comprising an active domain beginning at residue number 6 of this sequence, i.e., omitting the N terminal CXXXX, or omitting any of the preferred specific combinations such as CKRHP (SEQ ID NO: 70), CRRKQ (SEQ ID NO: 71), CKRHE (SEQ ID NO: 72), etc, resulting in a construct having only 6 cysteine residues. After this work, PCT 87/01537 was published, and it was observed that the proteins there identified as BMPII a and b and BMPIII each comprised a region embodying this generic structure. These proteins were not demonstrated to be osteogenic in the published application. However, applicants discovered that a subpart of the amino acid sequence of these protein, properly folded, and implanted as set forth herein, is active. These are disclosed herein as CBMPIIa, CBMPIIb, and CMBPIII. Also, the OP1 protein was observed to exhibit the same generic structure.

Please replace the first full paragraph on page 63, line 21, with the following paragraph.

Thus, the preferred osteogenic proteins are expressed from recombinant DNA and comprise amino acid sequences including any of the following sequences Vgl (SEQ ID NO: 7), DPP (SEQ ID NO: 8), OP1 (SEQ ID NO: 39), OP1 (SEQ ID NO: 9), CBMP-2a (SEQ ID NO: 10), CBMP-2b (SEQ ID NO: 11), CBMP-3 (SEQ ID NO: 12), COP1 (SEQ ID NO: 13), COP3 (SEQ ID NO: 14), COP4 (SEQ ID NO: 15), COP5 (SEQ ID NO: 1), COP7 (SEQ ID NO:2), and COP16 (SEQ ID NO: 16):

Please replace the sixth full paragraph on page 65, line 33, with the following paragraph.

As shown in FIGURE 18, these sequences have considerable homology with the alpha and beta inhibins (SEQ ID NOS: 52, 46 and 47, respectively), three forms of TGF beta (SEQ ID NOS: 48, 49, and 50, respectively), and MIS (SEQ ID NO: 51).

#### REMARKS

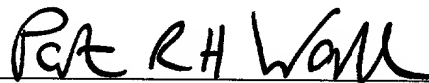
Claims 1-45 and 50-80 are canceled without prejudice and without any intention to abandon the subject matter claimed therein. Indeed, Applicants may still pursue claims of equal, lesser or greater scope. No new matter has been added by this amendment.

Claims 46-49 and the specification are amended to introduce sequence identifiers. Applicants respectfully submit that no new matter is introduced with this amendment.

A marked up version of the amended claims and a marked up version of the replacement paragraphs showing amendments are attached. A petition and fee for a four month extension of time up to and including September 10, 2001, is submitted herewith (September 8, 2001 was a Saturday). In the event that any additional fees are due, the Commissioner is hereby authorized to charge any such fees to Attorney's Deposit Account No. 20-0531.

Applicants respectfully submit that all of the pending claims are in condition for allowance and request early favorable action. If, in the Examiner's opinion, a telephonic interview would expedite allowance of the claims, the undersigned agent invites the Examiner to call him at the telephone number given below.

Respectfully submitted,



Patrick R. H. Waller, Ph.D.  
Agent for Applicant(s)  
Testa, Hurwitz, & Thibault, LLP  
High Street Tower  
125 High Street  
Boston, Massachusetts 02110

Date: September 10, 2001  
Reg. No. 41,418

Tel. No.: (617) 248-7240  
Fax No.: (617) 248-7100

09/10/2001 12:45:45

## MARKED-UP VERSION OF SPECIFICATION SHOWING AMENDMENTS

Replacement paragraph for the first full paragraph on page 8, line 1.

A series of consensus DNA sequences were designed with the goal of producing an active osteogenic protein. The sequences were based on partial amino acid sequence data obtained from the natural source product and on observed homologies with unrelated genes reported in the literature, or the sequences they encode, having a presumed or demonstrated developmental function. Several of the biosynthetic consensus sequences have been expressed as fusion proteins in procaryotes, purified, cleaved, refolded, combined with a matrix, implanted in an established animal model, and shown to have endochondral bone-inducing activity. The currently preferred active totally biosynthetic proteins comprise two synthetic sequences designated COP5 (SEQ ID NO: 1) and COP7 (SEQ ID NO: 2). The amino acid sequences of these proteins are set forth below.

Replacement paragraph for the third paragraph starting on page 9, line 23, and ending on page 10, line 8.

In one preferred aspect, these proteins comprise species of the generic amino acid sequences (SEQ ID NO: 3 and SEQ ID NO: 4, respectively):

```

1           10           20           30           40           50
  LXVXFDXGWXXWXXXPXGXXAXYCXXGXCXXPXXXXXXXXNHAXX
           60           70           80           90          100
QXXVXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXXVXLXXYXXMXVXXCXCX

```

or

```

1           10           20           30           40           50
CXXXXLXVXFDXGWXXWXXXPXGXXAXYCXXGXCXXPXXXXXXXXNHAXX
           60           70           80           90          100
QXXVXXNXXXXPXXCCXPXXXXXXXXLXXXXXXXXVXLXXYXXMXVXXCXCX

```

where the letters indicate the amino acid residues of standard single letter code, and the Xs represent amino acid residues. Preferred amino acid sequences within the foregoing generic sequences are (SEQ ID NO: 5 and SEQ ID NO: 6, respectively):

09754831-091001  
100760-TEST-260

Replacement paragraph for the second full paragraph on page 11, line 8.

Particular useful sequences include Vgl (SEQ ID NO: 7), DPP (SEQ ID NO: 8), OP1 (SEQ ID NO: 9), CBMP-2a (SEQ ID NO: 10), CBMP-2b (SEQ ID NO: 11), CBMP-3 (SEQ ID NO: 12), COP1 (SEQ ID NO: 13), COP3 (SEQ ID NO: 14), COP4 (SEQ ID NO: 15), and COP16 (SEQ ID NO: 16):

Replacement paragraph for the second full paragraph on page 14, line 5.

Analysis of digestion fragments indicate that the native 30 kD osteogenic protein contains the following amino acid sequences (question marks indicate undetermined residues):

- (1) S-F-D-A-Y-Y-C-S-G-A-C-Q-F-P-M-P-K (SEQ ID NO: 17);
- (2) S-L-K-P-S-N-Y-A-T-I-Q-S-I-V (SEQ ID NO: 18);
- (3) A-C-C-V-P-T-E-L-S-A-I-S-M-L-Y-L-D-E-N-E-K (SEQ ID NO: 19);
- (4) M-S-S-L-S-I-L-F-F-D-E-N-K (SEQ ID NO: 20);
- (5) S-Q-E-L-Y-V-D-F-Q-R (SEQ ID NO: 21);
- (6) F-L-H-C-Q-F-S-E-R-N-S (SEQ ID NO: 22);
- (7) T-V-G-Q-L-N-E-Q-S-S-E-P-N-I-Y (SEQ ID NO: 23);
- (8) L-Y-D-P-M-V-V (SEQ ID NO: 24);
- (9) V-G-V-V-P-G-I-P-E-P-C-C-V-P-E (SEQ ID NO: 25);
- (10) V-D-F-A-D-I-G (SEQ ID NO: 26);
- (11) V-P-K-P-C-C-A-P-T (SEQ ID NO: 27);
- (12) I-N-I-A-N-Y-L (SEQ ID NO: 28);
- (13) D-N-H-V-L-T-M-F-P-I-A-I-N (SEQ ID NO: 29);
- (14) D-E-Q-T-L-K-K-A-R-R-K-Q-W-I-?-P (SEQ ID NO: 30);
- (15) D-I-G-?-S-E-W-I-I-?-P (SEQ ID NO: 31);
- (16) S-I-V-R-A-V-G-V-P-G-I-P-E-P-?-?-V (SEQ ID NO: 32);
- (17) D-?-I-V-A-P-P-Q-Y-H-A-F-Y (SEQ ID NO: 33);
- (18) D-E-N-K-N-V-V-L-K-V-Y-P-N-M-T-V-E (SEQ ID NO: 34);
- (19) S-Q-T-L-Q-F-D-E-Q-T-L-K-?-A-R-?-K-Q (SEQ ID NO: 35);
- (20) D-E-Q-T-L-K-K-A-R-R-K-Q-W-I-E-P-R-N-?-A-R-R-Y-L (SEQ ID NO: 36);
- (21) A-R-R-K-Q-W-I-E-P-R-N-?-A-?-R-Y-?-?-V-D (SEQ ID NO: 37); and
- (22) R-?-Q-W-I-E-P-?-N-?-A-?-?-Y-L-K-V-D-?-A-?-?-G (SEQ ID NO: 38).

100160 TEST 260

Replacement paragraph for the second full paragraph on page 16, line 5.

The consensus DNA sequences are also useful as probes for extracting genes encoding osteogenic protein from genomic and cDNA libraries. One of the consensus sequences has been used to isolate a heretofore unidentified genomic DNA sequence, portions of which when ligated encode a protein having a region capable of inducing endochondral bone formation. This protein, designated OP1, has an active region having the sequence set forth below (SEQ ID NO: 39 and SEQ ID NO: 9, respectively).

Replacement paragraph for the fifth full paragraph on page 16, line 29.

Fig. 1A discloses the genomic DNA sequence of OP1 (SEQ ID NO: 40).

Replacement paragraph for the second full paragraph on page 18, line 7.

FIGURE 1A represents the nucleotide sequence of the genomic copy of osteogenic protein "OP1" gene (SEQ ID NO: 40). The unknown region between 1880 and 1920 actually represents about 1000 nucleotides;

Replacement paragraph for the third full paragraph on page 18, line 11.

FIGURE 1B is a representation of the hybridization of the consensus gene/probe (SEQ ID NO: 41) to the osteogenic protein "OP1" gene (SEQ ID NO: 42);

Replacement paragraph for the third full paragraph on page 20, line 14.

FIGURE 13 is a schematic representation of the DNA sequence (SEQ ID NO: 43) and corresponding amino acid sequence (SEQ ID NO: 44) of a consensus gene/probe for osteogenic protein (COP1);

Replacement paragraph for the second full paragraph on page 21, line 7.

FIGURE 18 is a comparison of the amino acid sequence of various osteogenic proteins to those of the TGF-beta family. COP1 (SEQ ID NO: 13), COP3 (SEQ ID NO: 14), COP4 (SEQ ID NO: 15), COP5 (SEQ ID NO: 1), and COP7 (SEQ ID NO: 2) are a family of analogs of

synthetic osteogenic proteins developed from the consensus gene that was joined to a leader protein via a hinge region having the sequence D-P-N-G (SEQ ID NO: 45) that permitted chemical cleavage at the D-P site (by acid) or N-G (by hydroxylamine) resulting in the release of the analog protein; VGI (SEQ ID NO: 7) is a Xenopus protein, DPP (SEQ ID NO: 8) is a Drosophila protein; OP1 (amino acids 6-x of SEQ ID NO: 9) is a native osteogenic protein; CBMP2a (SEQ ID NO: 10) and 2b (SEQ ID NO: 11), and CBMP3 (SEQ ID NO: 12) are subparts of proteins disclosed in PCT application 087/01537; beta-Inhibin a is shown in SEQ ID NO: 46, beta-Inhibin b is shown in SEQ ID NO: 47, TGF-beta 1 is shown in SEQ ID NO: 48, TGF-beta 2 is shown in SEQ ID NO: 49, TGF-beta 3 is shown in SEQ ID NO: 50; MIS (SEQ ID NO: 51) is Mullerian inhibitory substance; alpha-Inhibin is shown in SEQ ID NO: 52; and "consensus choices" represent various substitutions of amino acids that may be made at various positions in osteogenic proteins;

Replacement paragraph for the second full paragraph on page 22, line 4.

FIGURE 21B is the DNA sequence (SEQ ID NO: 53) and amino acid sequence (SEQ ID NO: 54) comprising a modified trp-LE leader, two Fb domains of protein A, an ASP-PRO cleavage site, and the COP5 sequence;

Replacement paragraph for the second paragraph starting on page 47, line 9, and ending on page 48, line 4.

Various of the peptide fragments produced using the foregoing procedures have been analyzed in an automated amino acid sequencer (Applied Biosystems 470A with 120A on-line PTH analysis). The following sequence data has been obtained:

- (1) S-F-D-A-Y-Y-C-S-G-A-C-Q-F-P-M-P-K (SEQ ID NO: 17);
- (2) S-L-K-P-S-N-Y-A-T-I-Q-S-I-V (SEQ ID NO: 18);
- (3) A-C-C-V-P-T-E-L-S-A-I-S-M-L-Y-L-D-E-N-E-K (SEQ ID NO: 19);
- (4) M-S-S-L-S-I-L-F-F-D-E-N-K (SEQ ID NO: 20);
- (5) S-Q-E-L-Y-V-D-F-Q-R (SEQ ID NO: 21);
- (6) F-L-H-C-Q-F-S-E-R-N-S (SEQ ID NO: 22);
- (7) T-V-G-Q-L-N-E-Q-S-S-E-P-N-I-Y (SEQ ID NO: 23);
- (8) L-Y-D-P-M-V-V (SEQ ID NO: 24);
- (9) V-G-V-V-P-G-I-P-E-P-C-C-V-P-E (SEQ ID NO: 25);



- (10) V-D-F-A-D-I-G (SEQ ID NO: 26);  
(11) V-P-K-P-C-C-A-P-T (SEQ ID NO: 27);  
(12) I-N-I-A-N-Y-L (SEQ ID NO: 28);  
(13) D-N-H-V-L-T-M-F-P-I-A-I-N (SEQ ID NO: 29);  
(14) D-E-Q-T-L-K-K-A-R-R-K-Q-W-I-?-P (SEQ ID NO: 30);  
(15) D-I-G-?-S-E-W-I-I-?-P (SEQ ID NO: 31);  
(16) S-I-V-R-A-V-G-V-P-G-I-P-E-P-?-?-V (SEQ ID NO: 32);  
(17) D-?-I-V-A-P-P-Q-Y-H-A-F-Y (SEQ ID NO: 33);  
(18) D-E-N-K-N-V-V-L-K-V-Y-P-N-M-T-V-E (SEQ ID NO: 34);  
(19) S-Q-T-L-Q-F-D-E-Q-T-L-K-?-A-R-?-K-Q (SEQ ID NO: 35);  
(20) D-E-Q-T-L-K-K-A-R-R-K-Q-W-I-E-P-R-N-?-A-R-R-Y-L (SEQ ID NO: 36);  
(21) A-R-R-K-Q-W-I-E-P-R-N-?-A-?-R-Y-?-?-V-D (SEQ ID NO: 37); and  
(22) R-?-Q-W-I-E-P-?-N-?-A-?-?-Y-L-K-V-D-?-A-?-?-G (SEQ ID NO: 38).

Replacement Table 6 on pages 54 and 55.

**TABLE 6**

<u>protein</u>	<u>amino acid sequence</u>	<u>homology</u>
(BOP) <u>SEQ ID NO: 55</u>	SFDAYYCSGACQFPS ***** * * *	(9/15 matches)
(DDP) <u>SEQ ID NO: 56</u>	GYDAYYCHGKCPFFL	
(BOP) <u>SEQ ID NO: 55</u>	SFDAYYCSGACQFPS * * * * *	(6/15 matches)
(Vgl) <u>SEQ ID NO: 57</u>	GYMANICYGECPYPL	
(BOP) <u>SEQ ID NO: 55</u>	SFDAYYCSGACQFPS * * * * *	(5/15 matches)
( <u>inhibin</u> ) <u>SEQ ID NO: 58</u>	GYHANYCEGECPSHI	
(BOP) <u>SEQ ID NO: 55</u>	SFDAYYCSGACQFPS * * * *	(4/15 matches)
( <u>TGF-beta</u> ) <u>SEQ ID NO: 59</u>	GYHANFCLGPCPYIW	
(BOP) <u>SEQ ID NO: 60</u>	K/RACCVPTELSAISMLYLDEN ***** * * * *	(12/20 matches)
(Vgl) <u>SEQ ID NO: 61</u>	LPCCVPTKMSPISMLFYDNN	

(BOP) SEQ ID NO: 60	K/RACCVPTELSAISMLYLDEN	
	* * * * *	(12/20 matches)
(inhibin) SEQ ID NO: 62	KSCCVPTKLRPMSMLYYDDG	

(BOP) SEQ ID NO: 60	K/RACCVPTELSAISMLYLDE	
	* * * *	(6/19 matches)
(TGF-beta) SEQ ID NO: 63	APCCVPQALEPLPIVYYVG	

(BOP) SEQ ID NO: 60	K/RACCVPTELSAISMLYLDEN	
	* * * * *	(12/20 matches)
(DPP) SEQ ID NO: 64	KACCVPTQLDSVAMLYLNDQ	

(BOP) SEQ ID NO: 65	LYVDF	
	* * * * *	(5/5 matches)
(DPP) SEQ ID NO: 66	LYVDF	

(BOP) SEQ ID NO: 65	LYVDF	
	* * *	(4/5 matches)
(Vgl) SEQ ID NO: 67	LYVEF	

(BOP) SEQ ID NO: 65	LYVDF	
	* * *	(4/5 matches)
(TGF-beta) SEQ ID NO: 68	LYIDF	

(BOP) SEQ ID NO: 65	LYVDF	
	* *	(2/4 matches)
(inhibin) SEQ ID NO: 69	FFVSF	

-  
\*-match

Replacement paragraph for the second paragraph starting on page 57, line 14, and ending on page 58, line 27.

Southern blot analysis of lambda #13 DNA showed that an approximately 3kb BamHI fragment hybridized to the probe. (See Fig. 1B). This fragment was isolated and subcloned into a bluescript vector (at the BamHI site). The clone was further analyzed by Southern blotting and hybridization to the COP0 probe. This showed that a 1kb (approx.) EcoRI fragment strongly hybridized to the probe. This fragment was subcloned into the EcoRI site of a bluescript vector,

and sequenced. Analysis of this sequence showed that the fragment encoded the carboxy terminus of a protein, named osteogenic protein-1 (OP1). The protein was identified by amino acid homology with the TGF-beta family. For this comparison cysteine patterns were used and then the adjacent amino acids were compared. Consensus splice signals were found where amino acid homologies ended, designating exon intron boundaries. Three exons were combined to obtain a functional TGF-beta-like domain containing seven cysteines. Two introns were deleted by looping out via primers bridging the exons using the single stranded mutagenesis method of Kunkel. Also, upstream of the first cysteine, an EcoRI site and an asp-pro junction for acid cleavage were introduced, and at the 3' end a PstI site was added by the same technique. Further sequence information (penultimate exon) was obtained by sequencing the entire insert. The sequencing was done by generating a set of unidirectionally deleted clones (Ozkaynak, E., and Putney, S.: Biotechniques, 5, 770-773, 1987). The obtained sequence covers about 80% of the TGF-beta-like region of OP1 and is set forth in FIG. 1A (SEQ ID NO: 40). The complete sequence of the TGF-beta like region was obtained by first subcloning all EcoRI generated fragments of lambda clone #13 DNA and sequencing a 4kb fragment that includes the first portion of the TGF-beta like region (third exon counting from end) as well as sequences characterized earlier. The gene on an EcoRI to PstI fragment was inserted into an *E. coli* expression vector controlled by the trp promoter-operator to produce a modified trp LE fusion protein with an acid cleavage site. The OP1 gene encodes amino acids corresponding substantially to a peptide found in sequences of naturally sourced material. The amino acid sequence of what is believed to be its active region is set forth below (SEQ ID NO: 39):

Replacement paragraph for the first full paragraph on page 59, line 1.

A longer active sequence is (SEQ ID NO: 9):

Replacement paragraph for the third paragraph starting on page 61, line 27, and ending on page 62, line 28.

It was noted, for example, that DPP from drosophila, VG1 from Xenopus, the TGF beta family of proteins, and to a lesser extent, alpha and beta inhibins, had significant homologies with certain of the sequences derived from the naturally sourced OP product. (Fig. 18.) Study of

these proteins led to the realization that a portion of the sequence of each had a structural similarity observable by analysis of the positional relationship of cysteines and other amino acids which have an important influence on three dimensional protein conformation. It was noted that a region of these sequences had a series of seven cysteines, placed very nearly in the same relative positions, and certain other amino acids in sequence as set forth below (SEQ ID NO: 4):

```

      10      20      30      40      50
CXXXXLXVFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXXNHAXX
      60      70      80      90     100
QXXVXXNXXXXPXXCCPXXXXXXXXLXXXXXXXXVXLXXYXXMXVXXCXCX

```

wherein each X independently represents an amino acid. Expression experiments with constructs patterned after this template amino acid sequence showed activity occurred with a shorter sequence having only six cysteines (SEQ ID NO: 3):

```

      10      20      30      40      50
LXVFXDXGWXXWXXXPXGXXAXYCXGXCXXPXXXXXXXXXNHAXX
      60      70      80      90     100
QXXVXXNXXXXPXXCCPXXXXXXXXLXXXXXXXXVXLXXYXXMXVXXCXCX

```

wherein each X independently represents an amino acid. Within these generic structures are a multiplicity of specific sequences which have osteogenic or chondrogenic activity. Preferred structures are those having the amino acid sequence (SEQ ID NO: 6):

```

      10      20      30      40      50
CKRHPLYVDFRDVGWNDWIVAPPGYHAFYCHGECFPFLADHLNSTNHAIV
RRRS K S S L QE VIS E FD Y E A AY MPESMKAS VI
KE F E K I DN L N S Q ITK F P TL
Q A S K

      60      70      80      90     100
QTLVNSVNP GKIPKACCVPTLSAISMLYLDENENVVLKKNYQDMVVEGCGCR
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H
RF T S K DPV V Y N S H RN RS
N S K P E

```

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be used. Novel active proteins also are defined by amino acid sequences comprising an active domain beginning at residue number 6 of this sequence, i.e., omitting the N terminal

03754831.091001

CXXXX, or omitting any of the preferred specific combinations such as CKRHP (SEQ ID NO: 70), CRRKQ (SEQ ID NO: 71), CKRHE (SEQ ID NO: 72), etc, resulting in a construct having only 6 cysteine residues. After this work, PCT 87/01537 was published, and it was observed that the proteins there identified as BMPII a and b and BMPIII each comprised a region embodying this generic structure. These proteins were not demonstrated to be osteogenic in the published application. However, applicants discovered that a subpart of the amino acid sequence of these protein, properly folded, and implanted as set forth herein, is active. These are disclosed herein as CBMPIIa, CBMPIIb, and CMBPIII. Also, the OP1 protein was observed to exhibit the same generic structure.

Replacement paragraph for the first full paragraph on page 63, line 21.

Thus, the preferred osteogenic proteins are expressed from recombinant DNA and comprise amino acid sequences including any of the following sequences Vgl (SEQ ID NO: 7), DPP (SEQ ID NO: 8), OP1 (SEQ ID NO: 39), OP1 (SEQ ID NO: 9), CBMP-2a (SEQ ID NO: 10), CBMP-2b (SEQ ID NO: 11), CBMP-3 (SEQ ID NO: 12), COP1 (SEQ ID NO: 13), COP3 (SEQ ID NO: 14), COP4 (SEQ ID NO: 15), COP5 (SEQ ID NO: 1), COP7 (SEQ ID NO:2), and COP16 (SEQ ID NO: 16):

Replacement paragraph for the sixth full paragraph on page 65, line 33.

As shown in FIGURE 18, these sequences have considerable homology with the alpha and beta inhibins (SEQ ID NOS: 52, 46, and 47, respectively), three forms of TGF beta (SEQ ID NOS: 48, 49, and 50, respectively), and MIS (SEQ ID NO: 51).

46. (Amended) A DNA sequence encoding an amino acid sequence sufficiently duplicative of that of the sequence encoded by the gene of Figure 1A[ ] (SEQ ID NO: 40) such that said encoded sequence induces bone or cartilage formation when implanted in a mammal in association with a matrix.

48. (Amended) The DNA sequence of claim 46 encoding:

49. (Amended) The DNA sequence of the claim 46 encoding:

2171990\_1